Short title: **Behaviour of moths in homogeneous clouds**

Flight behaviour of males of two moths, *Cadra cautella* and *Pectinophora gossypiella*, in homogeneous clouds of pheromone.

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Abstract. It is thought that orientation by male moths along pheromone plumes is guided by interception of filaments of pheromone along that plume and that clean air gaps are required for upwind progress. Given that several investigations have determined that cells sensitive to pheromone can resolve only low rates of encounter with pheromone filaments, generally less up to 10 pulses s^{-1}, it would appear that individual filaments encountered at higher rates would not be resolved by the insects' sensory system and, therefore, the stimulus would be perceived as a non-flickering signal. Behaviourally, this has been thought to be expressed as the cessation of upwind progress. Previous studies with *Cadra cautella* (Walker) (Lepidoptera: Pyralidae) demonstrated that upwind flight by these males is not inhibited in rapidly pulsed plumes. Therefore, to determine whether a flickering signal is required for upwind progress by *C. cautella*, males were introduced to homogeneous clouds of pheromone in a wind tunnel and their behaviour recorded. For comparison, *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae), a species from a long-diverged lineage, was also used. Upwind progress by *C. cautella* is not impeded by the constant olfactory signal provided by a homogeneous cloud of pheromone, but this is not true for *P. gossypiella*. Further, although *C. cautella* directs its flight upwind in a homogeneous cloud, its heading is not always due upwind. Potential mechanisms are discussed. It is suggested that *C. cautella* does not require a flickering signal to progress upwind.

Key words. *Cadra cautella*, *Pectinophora gossypiella*, pheromone, plume, anemotaxis, attraction

Introduction

Male moths navigate along airborne plumes of species-specific sex pheromones to locate mates. In-flight orientation along the plume is directed by optomotor anemotaxis (Kennedy & Marsh, 1974), but modulated by moment-to-moment contact with the odour plume (Baker, 1990; Kaissling & Kramer, 1990). A moth in flight directs its course angle more toward due upwind upon interception of pheromone, whereas loss of contact with the odour signal results in a more crosswind heading. Rate of turning and zigzagging by *Grapholita molesta* is modulated by pheromone concentration (Kuenen & Baker, 1982) and plume structure (Willis & Baker, 1984), and fine-scale structure of the plume has demonstrable effects on *Cadra cautella* (Walker) flight manoeuvres (Mafra-Neto & Cardé, 1994; 1995a,b). In both species, zigzag flights occur along ribbon-like plumes and straighter flights along turbulent plumes (Willis & Baker, 1984; Mafra-Neto & Cardé, 1995a), such that a moth receives an alternating signal of pheromone and air. Similarly, *Heliothis virescens* males flew straighter upwind in plumes pulsed at 4 s^{-1} than in plumes with lower pulse rates (Vickers & Baker, 1996).

Airborne odour plumes are subject to molecular and turbulent diffusion, the effect of which is to produce a sinuous plume with a filamentous internal structure. The instantaneous structure of a plume, as measured by a small sensor with high frequency response, can be described as a series of bursts of odour that vary in concentration, duration, and rate of repetition (Murlis & Jones, 1981; Murlis *et al.*, 2000).

The ability of a moth to resolve a flickering signal of this kind is a product of components at each level of sensory processing. Receptors, sensory cells, antennae, and successive levels within the central nervous system show characteristic responses to a fluctuating signal, and may or may not 'perceive' it as intermittent. Two sensory cell types of *Antheraea polyphemus* that respond to the two minor components of that insect's sex pheromone, resolve pulses as brief as 20 ms at rates of approximately 5 Hz, whereas cells responding to the major component resolve pulses of only 2 Hz (Rumbo & Kaissling, 1989). Cells of *Manduca sexta* are capable of resolving 20 ms pulses of bombykol at up to 3 Hz (Marion-Poll & Tobin, 1992). Likewise, most projection

neurones in the macroglomerular complex of this species can resolve 25 ms pulses of pheromone at the antenna at a rate of up to 4 Hz, with some being able to follow pulses as frequent as 10 Hz (Christensen & Hildebrand, 1988). Lei & Hansson (1999) found that interneurones in the antennal lobe (AL) of *Agrotis segetum* possess differing properties in which some neurones reflect the temporal features of the plume impinging on the antenna, at least to 5 Hz.

A plume that provides an intermittent signal with a pulse frequency that exceeds the response rate of an insect's neural circuitry should be perceived essentially the same as a single, long-duration pulse of stimulus. Clouds of pheromone that lacked notable pockets of clean air produced an arrestment of upwind flight by *Adoxophyes orana* (Kennedy *et al.*, 1980; 1981) and *G. molesta* (Willis & Baker, 1984). Presumably, a 'fused signal' (*i.e.* a signal pulsed at a rate that exceeds the insect's neural response rate) would produce a behaviour similar to that of an insect in a non-flickering signal, expressed as a lack of progress in upwind flight.

A previous study (Justus *et al.*, 2002) demonstrated that *C. cautella* flies upwind when presented with a flickering signal delivered at 25 Hz. Because a moth flies into the wind at airspeeds up to 1.2 m s^{-1}, the rate of flicker encounter (*i.e.* realized rate of encounter) is in excess of 60 Hz. Given the neurophysiological evidence from other aforementioned species, it seems unlikely that an insect could resolve that rate of flicker. We postulated instead that *C. cautella* does not require a flickering signal to orient upwind in an odour plume. We investigated that possibility using a homogeneous pheromone cloud, devoid of fine-scale structure and intermittency -- a truly non-flickering signal.

Materials and Methods

Insects:

A colony of *C. cautella* was reared from eggs to last-instar larvae in 1-L jars containing rolled oats, brewer's yeast and glycerine (see Mafra-Neto & Cardé, 1995a). For experiments, last-instar

larvae were removed from the colony and sexed. Males were placed in plastic containers (6 x 18 x 12 cm) held in an environmental growth chamber at 25 ° C, 16:8 L:D and ~ 65% R.H., and allowed to pupate. Pupae of *Pectinophora gossypiella* (Saunders) were obtained from USDA/APHIS at Phoenix, Arizona. Male pupae were held singly in shell vials in an environmental growth chamber as above. For each species, adults were transferred to a 30 x 30 x 30 cm plastic screened cage and held in a growth chamber (as above) with access to 10% sucrose. Animals tested were 2 - 3 days post-eclosion.

Wind tunnel:

The wind tunnel comprised four sheets of 60 x 180 cm Plexiglas® held together with clear acrylic tape to form a rectangular (60 x 60 x 180 cm) tunnel. A 15 x 23 cm opening was cut into the downwind end on one side to allow access to the tunnel. At the upwind end of the tunnel, an aluminum frame screened with 3.5 mm wire mesh formed the grid for the odour delivery tubes. To promote mixing, three baffles of screened (1 mm mesh) aluminum frames and masking tape (4 cm wide, 4 cm apart), were set into the upwind end of the tunnel as follows: horizontal strips only, set 12 cm into the tunnel; vertical strips only at 24 cm; and both horizontal and vertical strips at 36 cm. Each was taped in place with transparent acrylic tape so that edges were sealed entirely, preventing entrainment of clean air. The last baffle was followed by a 6 cm space and a 15-cm deep section of aluminum honeycomb (Hexel®; 1.5 cm diam cells). A polyurethane cowling connected the downwind end of the tunnel to an exhaust system.

This entire structure was set into a semi-cylindrical wind tunnel (see Justus *et al.*, 2002), because the airflow produced by its upwind fan aided our ability to produce a uniform cloud of pheromone with a laminar flow.

The tunnel was housed in a room with a controlled environment at 26-28 °C and 60-70% R.H. Two banks of 25 watt clear red (Philips Colortone®) and white incandescent lights were arranged on either side of the room and illuminated the ceiling. Two infrared LED arrays (940 nm; TrackSys, UK) to supplement light for video recording were placed at the downwind end of the

tunnel. Light levels inside the wind tunnel were 4 - 8 lux (with lower light levels used for *P. gossypiella*). A floor pattern consisted of randomly placed but non-overlapping red paper dots (6 cm diameter) at a density of 60 dots m^{-2}, on a white floor. To minimize external visual cues, white fabric completely covered the outer tunnel.

Odour sources:

A glass basin 25 cm x 16 cm diameter with a 2 cm constriction at 12 cm height was fabricated. Above that constriction the basin expanded to 20 cm diameter. Two opposing barbed inlets were added 5 cm from the bottom of the basin. A Teflon® plate (1 cm thick), with 121 holes (5.5 mm diameter) equidistant from each other, covered the basin and was secured with a latex O-ring between the basin and Teflon plate, and a metal spring-type O-clamp. Teflon tubes (4.7 cm inner diameter) 60 cm long were strung from the top of the Teflon plate into the 3.5 mm wire grid at the opening of the tunnel.

At the outset of each experiment, 1, 5, 16 or 50 rubber septa each containing 1 mg of synthetic *C. cautella* sex pheromone (9:1 ratio of (*Z,E*)-9,12-tetradecadienyl and (*Z*)-9-tetradecenyl acetates) or 4 mg of *P. gossypiella* sex pheromone (1:1 ratio of (*Z,Z*)-7,11- and (*Z,E*)-7,11-hexadecadienyl acetates) were suspended via copper wires and hung from a small metal grid across the constriction in the glass basin. For *C. cautella*, solutions of pheromone were formulated gravimetrically from synthetic pheromone components (Bedoukian Research, Inc., USA) and diluted to 10 µg $µl^{-1}$. Septa were loaded with 100 µl of the binary blend, and aired overnight in a fume hood. Septa loaded with the sex pheromone components of *P. gossypiella* were obtained from the USDA/APHIS, Arizona (see Flint *et al.*, 1978, for a description of preparation method). Pheromone-loaded septa were held at -28 ° C until use. So that the basin always had a total of 50 septa, blank septa were used to make-up the difference in numbers. A magnetic stirring bar was used to agitate compressed air (3 L min^{-1}) which entered the basin through the opposing barbed inlets forcing air up and past the pheromone-loaded septa. To

ensure a uniform concentration of pheromone in the tunnel, the system was allowed to equilibrate for 2 h before experiments began.

Plume structure:

Because it is not feasible to measure the homogeneity of a pheromone cloud directly, propylene was used as a surrogate odour and a miniature photoionization detector (miniPID; Aurora Scientific Inc., Canada) assessed the spatial and temporal uniformity of the concentration. A propylene cloud was created using the same odour delivery apparatus, except that blank septa were placed in the glass basin and a 1:1 mixture of compressed air and propylene entered the inlets of the basin. At a 3 L min^{-1} flow rate and a tunnel speed of 50 cm s^{-1}, this resulted in an equilibrium in the tunnel of ~140 ppm.

To determine the concentration and variability within the surrogate cloud, the miniPID was mounted on a custom-made, motorized, computer-controlled x,y,z-traverse. This allowed the miniPID to be positioned at any point in the tunnel. Concentrations at points along the x-, y-, and z-axes of the tunnel were sampled for 30 s at a rate of 100 Hz and recorded digitally using GemAcq4 (Gemini Enterprises, UK) software.

Assays:

At least 40 males of each species were tested for each treatment. Moths were held one at a time in a cylindrical cage (6 cm x 3.5 cm diameter) made of metal screening (1 mm mesh) with the open end of the cylinder facing downwind, 1 m downwind from the screen at the downwind side of the Hexel laminizer. When a moth started wing fanning, the cage was rotated 180° allowing the moth to exit upwind. Time to fanning and takeoff were noted.

Flight tracks were recorded with a CCD video camera (Sanyo VCB-3512T; shutter speed 1/250; equipped with a 6.0 mm lens) mounted above the wind tunnel to allow coverage of the entire working section of the tunnel (*c.* 0.75 m^2), and a Sony GV-A500 Hi8 video recorder.

Video and analyses:

Flight tracks were digitized at 15 frames s^{-1} (66.7 ms) using Motus® (Peak Performance Technologies Inc., USA) software. For each vector within each track, 2-D parameters of ground speed, airspeed, upwind and crosswind speeds, and track and course angles were calculated (see Charlton *et al.*, 1993, for definitions of these parameters). To assess more general aspects of flight tracks, the number of moths flying upwind and landing at the upwind screen, the time to wing fanning and takeoff, and the elapsed time between takeoff and landing were recorded for each flight.

Vector-by-vector analyses were carried out only for track and course angles and absolute values of vectors were used to calculate means of each. For parametric procedures, the mean values of ground speed, upwind speed, crosswind speed and airspeed of each track were subjected to an analysis of variance.

Results

Plume Structure:

The surrogate cloud produced was found to have a peak-to-mean ratio approaching 1.0 throughout. The mean concentration along the x-axis at > 250 mm downwind of the Hexel showed a 2 % variation, but almost no variability upwind of that point (Fig. 1). This variability in concentration appeared to be due to a slight waiver in airflow at the downwind end of the tunnel, caused by the exhaust system. Concentration was consistent along the y-axis (*i.e.* left and right of the longitudinal mid-line) and the z-axis (*i.e.* the vertical axis).

Assays:

No moths wing fanned at the lowest concentration (1 septum) but a small percentage did when 5 septa were used, and 100% at the mid- and high-concentrations (16 and 50 septa, respectively). Time to wing fanning was highly variable between males (Table 1), though male *P. gossypiella*

took longer to initiate wing fanning. No moths of either species initiated flight at the lower pheromone concentrations of 1 or 5 septa. However, most males initiated flight when moderate (16 septa) or high concentrations (50 septa) were used (Table 1). The percentages of male *C. cautella* that flew upwind were 83 and 100%, for moderate and high concentrations respectively; but fewer *P. gossypiella* flew upwind (4 and 13%). Percentages of moths landing at the upwind screen, a distance of approximately 1 m, were only slightly lower. Time elapsed from takeoff to landing was longer for *C. cautella* than for *P. gossypiella* (Fig. 2a), with any cloud concentration there were no differences within species in the amount of time elapsed.

Analysis of flight parameters show that track and course angles were larger for *P. gossypiella* than *C. cautella* and this was evident in individual flight tracks, with *C. cautella* exhibiting upwind headings (Fig. 2b,d) and *P. gossypiella* tending to spiral and then land immediately (Fig. 2c,e). Because very few males of *P. gossypiella* headed upwind, the flight direction was checked to see if it was random by defining simple flight vectors as being from the origin (*i.e.*, the release cage) to first landing (Fig. 3). Vectors were applied to a circular grid consisting of 9 sections spanning 40 ° each (with –20 ° to 20 ° being the trajectory to the outer edges of the upwind screen). These 'simple vectors' demonstrated that *C. cautella* had a 'preferred' upwind heading with nearly all vectors in this section of the grid (two way ANOVA; $\alpha = 0.05$; $P < 0.0001$), but *P. gossypiella* showed no directional 'preference'.

There were no differences in heading and velocity between 16- and 50-septa pheromone clouds for *P. gossypiella*, but *C. cautella* did exhibit differences between cloud concentrations. Mean track and course angles were aimed more toward crosswind at the high concentration and upwind speed was slower (Fig. 4). However, using the simple vectors (Fig. 3), we also calculated deviation from wind line and found no differences (Mann-Whitney Rank Sums test) between *C. cautella* males provided with either the moderate- or high-concentration pheromone cloud (Table 2).

Discussion

Kennedy *et al.* (1980, 1981) and Willis & Baker (1984) found that the tortricid moths *A. orana* and *G. molesta*, respectively, did not progress upwind in pheromone clouds. In both cases, fluctuations in pheromone signal were assumed to be necessary for upwind progress; continuous stimulation of sensory receptors provided by a pheromone cloud was considered to be an inadequate stimulus to sustain upwind flight. Moths were initially presented with a plume from a point source, which was removed once the moth was in flight, and then replaced with a well mixed cloud of pheromone or 'miasma'. In both studies, clouds were checked subjectively for homogeneity using $TiCl_4$ 'smoke' as a visual marker. However this, by its nature, could have obscured pockets of lower concentrations or of clean air. In a subsequent experiment using a similar apparatus to generate a pulsed cloud, Baker *et al.* (1985) observed variability in density within the cloud using an infrared diode and photometer.

The procedures with *C. cautella* and *P. gossypiella* employed here differ from the studies with *A. orana* and *G. molesta* in two ways. First, a miniPID was used to measure the homogeneity of the miasma quantitatively, and this confirmed that our setup reliably produced a truly homogeneous cloud. Second, point-source plumes were not used to initiate upwind flight.

Both species wing fan at the mid- and high-concentration clouds, but wing fanning is rare at the lower concentrations. The greater latency of wing fanning on average for *P. gossypiella* than for *C. cautella* could be due to an overall relatively lower concentration of pheromone, given the lower volatility of the 16-carbon acetates of *P. gossypiella*, despite the 4-fold higher dose on each septum, or it could be simply a species effect. Collins & Cardé (1989) reported highly variable and long latencies to wing fanning by *P. gossypiella* over a range of pheromone concentrations (produced from 10 ng to 10^{-3} ng of pheromone on a filter paper disk). In that study, mean wing-fanning latencies were between 40 and 108 s; shorter latencies occurred with increased pheromone concentration. Latencies recorded in the current study are similar to those reported by Collins & Cardé for 10 ng filter paper sources and, therefore, the actual concentration

of a pheromone cloud produced from 16 septa (*i.e.* our mid-concentration) should be similar to pheromone plumes issuing from 10 ng sources of the previous study.

There are obvious contrasts in flight behaviours between *C. cautella* and *P. gossypiella* in homogeneous clouds. *Pectinophora gossypiella*, like *A. orana* and *G. molesta*, do not fly upwind in a homogeneous cloud. However, unlike *A. orana* and *G. molesta*, *P. gossypiella* do not cast under these circumstances, but rather land within 2 s of takeoff in mid- and high-concentration pheromone clouds. It is not known if this rapid cessation of flight is due to our experimental protocol of not introducing *P. gossypiella* first to a point source plume for flight initiation, so that a casting behaviour would ensue upon removal of the point-source, or if it is due to an 'overloading' of sensory receptors (addressed later in the text). In addition, the direction of *P. gossypiella* upon takeoff is random, which indicates that takeoff itself was not anemotactic, and heading is not corrected in-flight, but rather flight ceases altogether. That they are not able to orient upwind suggests that, in *P. gossypiella*, a plume without a fluctuating signal is sufficient to initiate flight but not to sustain it.

Conversely, *C. cautella* progresses upwind in a homogeneous cloud and, therefore, it can be concluded that: a fluctuating signal is not required for *C. cautella* to initiate and maintain upwind progress, nearly all moths tested at moderate and high concentrations leave the release cage and fly upwind; self-steered counterturns (as shown in Fig. 2d) are not dependent upon signal fluctuations because the pheromone cloud lacks such fluctuations, especially within 250 mm from the upwind screen, an area in which counterturns frequently occur; upwind progress is maintained to 'source', in this case a 60 x 60 cm screen that does not provide a single focal point as a visual cue; and there is some effect of concentration, because male *C. cautella* fly more nearly due upwind at the moderate concentration than at the high concentration. However, upwind heading at both concentrations is rarely due upwind and counterturning is exhibited even in the absence of a plume edge.

Mafra-Neto & Cardé (1995a) demonstrated that *C. cautella* performs different flight manoeuvres in distinctly different types of plume (ribbon and turbulent plumes): evidence that

flickering signals and the pulse frequency of that signal (*i.e.*, rate of flicker) largely determines the shape of the flight track. Zigzag tracks occur when a moth is presented with a ribbon plume in which a moth intersects the plume and turns back to intersect the plume again at some distance upwind. Essentially, the antennae receive an alternating signal, just as they would in the much straighter flight tracks of moths in turbulent or pulsed plumes that comprise filaments of odour separated by clean air. Justus *et al.* (2002) found that even in very rapidly pulsed plumes of 25 Hz, track angles were toward due upwind, and similar to what are reported here for homogeneous plumes. However, the ground speed at which moths travel in homogeneous plumes is much slower than in rapidly pulsed plumes. Ground speed has been shown to vary with pheromone concentration (Cardé & Hagaman, 1979), and it is probable that the homogeneous cloud was of a lower concentration than the pulsed plume of Justus *et al.* (2002). Regardless, the results in this study and in Justus *et al.* (2002) suggest that *C. cautella* has a second mechanism enabling it to progress upwind in plumes that flicker so rapidly that air-gaps are undetectable. A flight program at an angle to the wind line (such as the 15 ° measured in *C. cautella* flights) would allow upwind progress and would also facilitate locating the edge of a plume (in this case, a non-existent circumstance).

Male *C. cautella* sustained flight for an average duration of 4 s. Although this is of short duration relative to field situations, it is of considerable length when one includes pre-flight exposure to a constant pheromone signal (approximately 30 s). When a moth is presented with a homogeneous cloud in which the signal is constant regardless of track, at the level of olfactory sensory cell, one of two possibilities is likely. The first of these is that a train of impulses is produced in which the frequency gradually or rapidly declines (*i.e.,* the cell adapts). High concentrations of pheromone (which generally have a large effect in sensory adaptation) can impair upwind progress, such as in *L. dispar* (Cardé & Hagaman, 1979; Charlton *et al.*, 1993) and *A. segetum* (Löfstedt *et al.*, 1985). Baker *et al.* (1989) reported that sensory cells of *A. segetum* do not show adaptation to low dispenser doses (sources of 0.3 - 30 µg) of Z5-10:Ac but do adapt at higher dispenser doses (300 µg). Vickers & Baker (1994) proposed that sustained upwind

flight is due to "fast-acting phasic-tonic surging-casting response system" in response to the pheromone filaments of a turbulent or pulsed plume that allows the cell to disadapt between pulses of stimulus. Kennedy *et al.* (1980, 1981), however, suggested that arrestment of upwind progress in homogeneous clouds is not the result of adaptation, but rather that the required stimulus for upwind flight is itself a flickering signal.

A second possibility is that the sensory cells produce a train of impulses in which the frequency does not decrease over time (*i.e.*, there is no sensory adaptation). Grant *et al.* (1989) reported that continuous stimulation (650 s) of olfactory neurones of *Trichoplusia ni* "exhibited relatively constant levels of neural activity throughout the stimulus period". Further, two of three types of pheromone component-specific cells in *A. segetum* did not show any adaptation to stimulations of 20 s duration (Hansson & Baker, 1991). Whether or not concentration of the stimulus has an effect with prolonged stimulation is unknown for both *C. cautella* and *P. gossypiella*. Although *A. segetum* has some cell types that do not show adaptation to prolonged stimulation (Hansson & Baker, 1991), Baker *et al.* (1989) reported that cells of *A. segetum* adapt to the *Z*5-10:Ac component with 20 s stimulation at higher concentrations. A comparison with *Heliothis virescens* in the same study shows no such adaptation to the same stimulation period with *Z*11-16:Ald, even at high concentrations. The concentrations in this study, including the highest, were described earlier as being relatively low, which would suggest the possibility that pheromone-sensitive cells of *C. cautella* do not adapt. However, concentrations used with *P. gossypiella* were similarly low so, without neurophysiological data, it is only possible to speculate on whether either of these two species have pheromone-sensitive cells that adapt with prolonged stimulation.

A homogeneous plume would not be expected in natural, open environments. However, *C. cautella* is a pest of stored products and therefore can occur in enclosed, often wind-free environments. In such a habitat, turbulence is greatly reduced or non-existent and the plume structure is more likely to be influenced by molecular diffusion where a cloud (albeit, not truly homogeneous) of low concentration could eventuate. A male's ability to locate a female in such

circumstances might depend on an insusceptibility to adaptation and an ability to navigate broad, low concentration odour plumes that have little internal structure. The hypothesis that this mechanism is adaptive for orientation in specific habitats could be tested by comparing *C. cautella* behaviours with that of taxonomically distant relatives that are also pests of stored products, such as *Sitotroga cerealella* (Gelechiidae), and related phycitines such as *Dioryctria* spp. that are not stored product pests. If a second mechanism to navigate structurally depauperate plumes is an adaptive strategy of stored product pests, it is more likely that distant relatives occurring in such habitats will show similar behaviours to *C. cautella* than sister species inhabiting only open environments.

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Table 1. Wing fanning, take-off and upwind flights by *C. cautella* and *P. gossypiella* males presented with homogeneous plumes of differing concentrations.

No. of septa	No. moths tested	% wing-fanning	Mean (±S.D.) time (s) to wing-fan	% take-off	% flights upwind	% landing at screen
***P. gossypiella*:**						
1	40	0.0	---	0.0	0.0	0.0
5	40	3.0	---	0.0	0.0	0.0
16	47	76.6	38.07 ± 26.46	72.3	4.3	2.1
50	56	92.9	23.33 ± 16.58	91.1	12.5	8.9
***C. cautella*:**						
1	40	0.0	---	0.0	0.0	0.0
5	40	5.0	7.50 ± 3.54	0.0	0.0	0.0
16	42	100.0	7.62 ± 11.00	92.8	83.3	78.5
50	41	100.0	5.72 ± 6.71	95.1	95.1	87.8

Table 2. Deviation* of *C. cautella* males from wind line (mean ± S.D.) using 'simple vectors' (see text).

cloud concentration	all vectors angle °	left of wind line angle °	right of wind line angle °
moderate (16 septa)	13.21 ± 10.38	10.58 ± 8.26	16.23 ± 11.92
high (50 septa)	15.06 ± 11.17	15.06 ± 11.43	15.64 ± 11.46

* deviations between absolute values of left and right ($P = 0.8890$), and between moderate- and high-concentration clouds ($P = 0.5394$) do not differ; Mann-Whitney Rank Sums.

Fig. 1. Propylene concentrations (V) measured along x-axis (*i.e.*, longitudinal axis) of the wind tunnel at the mid-line of y- (horizontal) and z- (vertical) axes using a mini photoionization detector. "X" values are successive points downwind from the upwind screen. Each was sampled at 100 Hz for 30 seconds. Dashed line represents miniPID measurement when no propylene was present.

Fig. 2. (a) Elapsed time (± S.E.) from takeoff to landing. *P. gossypiella* flights are of significantly shorter durations than those of *C. cautella* (Kruskal-Wallis; *alpha* = 0.05, $P < 0.0001$), but no differences exist within species. Representative flight tracks, selected using survivorship analysis, with upwind headings of (b & d) *C. cautella* and (c & e) *P. gossypiella* in a homogeneous cloud generated from 16 (b & c) and 50 septa (d & e).

Fig. 3. 'Simple flight vectors' of *C. cautella* in a homogeneous cloud generated from (a) 16 and (b) 50 septa, and of *P. gossypiella* in a homogeneous cloud generated from (c) 16 and (d) 50 septa. Each arrow represents one flight from the release cage (origin) to first landing (arrowhead). Dashed lines are trajectories from release cage to the outer edges of the upwind screen (grey trapezoid); these trajectories indicate the 'upwind section' of the grid used in a two way ANOVA (see text). Note that the grey screen does not appear rectangular due to the three-dimensionality of the wind tunnel presented in a two dimensional perspective (*i.e.* the top of the screen is nearest the camera and is therefore larger than the bottom).

Fig. 4. Means (± S.E.) for flight parameters of *C. cautella*. Letters indicate significant differences; Kruskal-Wallis; *alpha* = 0.05, $P < 0.001$

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